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Filed on 15 October 2003 (15.10.2003)

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 23 March 2006

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MODULATION OF APOLIPOPROTEIN (A) EXPRESSION

(57) Abstract: Compounds, compositions and methods are provided for modulating the expression of apolipoprotein(a). The compositions comprise oligonucleotides, targeted to nucleic acid encoding apolipoprotein(a). Methods of using these compounds for modulation of apolipoprotein(a) expression and for diagnosis and treatment of disease associated with expression of apolipoprotein(a) are provided.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/14540

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12Q 1/68; A01N 43/04; C07H 21/04; A61K 31/07
US CL : 435/6, 91.1, 325, 375; 536/23.1, 24.3, 24.33, 24.5, 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/6, 91.1, 325, 375; 536/23.1, 24.3, 24.33, 24.5, 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BRANCH, AD. A good antisense molecule is hard to find. TIBS, 1998 Vol. 23:45-50, see entire article.	26-28, 32-36, and 43-49
A	JEN et al. Suppression of gene expression by targeted disruption of messenger RNA: Available options and current strategies. Stem Cells, 2000 Vol. 18:307-319, see entire	26-28, 32-36, and 43-49
X	MORISHITA et al. Novel therapeutic strategy for atherosclerosis ribozyme oligonucleotides against apolipoprotein(a) selectively inhibits apolipoprotein (a) but not plasminogen gene expression. Circulation, 1998 Vol. 98:1898-1904, see page 1899, first column.	18, 26, 27, 31, 34, 35, 37, 47-49
X	MCLEAN et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature, 1997 Vol. 330:132-137, see Figure 1b at dotted underline.	18, 26, 27, 31, 34, 35, 37, 47-49
X	US 6,008,344 (BENNETT et al.) 23 February 1999 (23.2.1999), see SEQ ID NO:43	18, 20-27, 31, 34-40, and 47-49
X	WO 99/35241 (PHARMACEUTICALS, INC.) 8 January 1998 (8.1.1999), see page 23, first full paragraph	18, 22-25, and 31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
06 December 2005 (06.12.2005)

Date of mailing of the international search report
25 JAN 2006

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (571) 273-3201

Authorized officer
Terra C. Gibbs
Telephone No. 571-272-0564

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/14540

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

a. type of material

☒ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☒ on paper

☒ in electronic form

c. time of filing/furnishing

☒ contained in the international application as filed

☒ filed together with the international application in electronic form

☐ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/14540

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-28, 30-49, and SEQ ID NO:85

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claims 1-28 and 31-49 drawn to compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) and a method of using said compound in cells or tissues comprising administering a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) or treating a disease or disorder associated with apolipoprotein (a) comprising administering a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a).

Group II, claim 29, drawn to a method of screening for a modulator of apolipoprotein (a).

Group III, claim 30, drawn to a diagnostic method for identifying a disease state.

The inventions listed as Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups II and III are each directed to different methods than the treatment methods in Group I. Methods of screening and methods of identifying are clearly different special technical features from the methods of treatment.

Claims 1, 19, and 28 are subject to an additional restriction since it is not considered to be a proper genus/Markush. If the members of the Markush group are sufficiently few in number or so closely related that a search and examination of the entire claim can be made without serious burden, the examiner must examine all the members of the Markush group in the claim on the merits, even though they are directed to independent and distinct inventions. In such a case, the examiner will not follow the procedure described below and will not require restriction. Broadly, unity of invention exists where compounds included within a Markush group (1) share a common utility, and (2) share a substantial structural feature disclosed as being essential to that utility.

Claims 1, 19, and 28 specifically claims antisense SEQ ID NOs. 85-96, 11, 23, 28, 30, 31, 33-36, 39, 42, 43, and 45, which are targeted to and modulate the expression of apolipoprotein (a). Although the antisense sequences claimed each target and modulate expression of apolipoprotein (a), the instant antisense sequences are considered to be unrelated, since each antisense sequence claimed is structurally and functionally independent and distinct for the following reasons: each antisense sequence has a unique nucleotide sequence, each antisense sequence targets a different and specific region of apolipoprotein (a) nucleic acid, and each antisense, upon binding to a apolipoprotein (a) nucleic acid, functionally modulates (increases or decreases) the expression of the gene and to varying degree (per applicants' Table 1 in the specification). As such, the Markush/genus of antisense sequences in claims 1, 19, and 28 is not considered to constitute a proper genus, and is therefore subject to restriction. Furthermore, a search of more than one (1) of the antisense sequences claimed in claims 1, 19, and 28 presents an undue burden on the Patent and Trademark Office due to the complex nature of the search and corresponding examination of more than one (1) of the claimed antisense sequences. In view of the foregoing, one (1) antisense sequence is considered to be a reasonable number of sequences for examination. Accordingly, applicants are required to elect one (1) antisense sequence from claims 1, 19, and 28. Note that this is not a species election.

Applicants will obtain a search of the first invention listed in the first group. For every other invention applicants wish to have searched, applicants need to elect the group and pay an additional fee. Additionally, applicants will obtain a search of the first sequence listed in

the first invention. For every other sequence applicants wish to have searched, applicants need to elect the sequence and pay an additional fee.

Continuation of B. FIELDS SEARCHED Item 3:
STN, WEST, NPL, Medline, CaPLUS, EmBase
search terms: antisense, ribozyme, apolipoprotein (a), plasminogen

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference ISPH-0595WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2004/014540	International filing date *(day/month/year)* 02 June 2004 (02.06.2004)	Priority date *(day/month/year)* 02 June 2003 (02.06.2003)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant ISIS PHARMACEUTICALS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 13 March 2006 (13.03.2006)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 740 14 35	Authorized officer Nora Lindner Telephone No. +41 22 338 89 65

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

REC'D 30 JAN 2006
WIPO PCT

To:	
MARY E. BAK HOWSON AND HOWSON 321 NORRISTOWN ROAD, SUITE 200, SPRING HOUSE CORPORATE CENTER, PO BOX 457 SPRING HOUSE, PA 19477	**PCT** WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43*bis*.1)
	Date of mailing (*day/month/year*) 25 JAN 2006
Applicant's or agent's file reference ISPH-0595WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US04/14540	02 June 2004 (02.06.2004)	02 June 2004 (02.06.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/68; A01N 43/04; C07H 21/04; A61K 31/07 and US Cl.: 435/6, 91.1, 325, 375; 536/23.1, 24.3, 24.33, 24.5, 514/44

Applicant

ISIS PHARMACEUTICALS, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [x] Box No. IV — Lack of unity of invention
- [x] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [x] Box No. VII — Certain defects in the international application
- [x] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis(b)* that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	07 December 2005 (07.12.2005)	Terra C. Gibbs Marina J Watson Telephone No. 571-272-0564

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US04/14540

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:
   - ☒ the international application in the language in which it was filed
   - ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
   - a. type of material
     - ☒ a sequence listing
     - ☐ table(s) related to the sequence listing
   - b. format of material
     - ☒ on paper
     - ☒ in electronic form
   - c. time of filing/furnishing
     - ☒ contained in the international application as filed.
     - ☒ filed together with the international application in electronic form.
     - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/14540

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
   - ☐ paid additional fees
   - ☐ paid additional fees under protest and, where applicable, the protest fee
   - ☐ paid additional fees under protest but the applicable protest fee was not paid
   - ☒ not paid additional fees
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
   - ☐ complied with
   - ☒ not complied with for the following reasons:

   See the lack of unity section of the International Search Report(Form PCT/ISA/210)

4. Consequently, this opinion has been established in respect of the following parts of the international application:
   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-28, 31-49, and SEQ ID NO:85

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/14540

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-17, 19, 28, 32, 33, 41, 42, and 43-46	YES
	Claims	18, 20-27, 31, 34-40, 47-49	NO
Inventive step (IS)	Claims	1-17, 19, 28, 32, 33, 41, 42, and 43-46	YES
	Claims	18, 20-27, 31, 34-40, 47-49	NO
Industrial applicability (IA)	Claims	1-49	YES
	Claims	NONE	NO

2. Citations and explanations:

Please See Continuation Sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US04/14540

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

Claim 43 is objected to under PCT Rule 66.2(a)(iii) as containing the following defect(s) in the form or contents thereof: Claim 43 recites, "said animal" in line 4. There is insufficient antecedent basis for this limitation in the claim because the claim refers to " a subject", not an animal.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the questions whether the claims are fully supported by the description, are made:

Please See Continuation Sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US04/14540

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:
Claims 1-49 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 1-17, 19, and 28 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a compound targeted to at least a portion of a nucleic acid molecule encoding apolipoprotein (a), wherein said compound is at least 70% complementary to said portion of said nucleic acid molecule encoding apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a), and wherein said compound is SEQ ID NO:85, and a method of using said compound in cells or tissues.

Claim 32, 33, and 41-46 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of administering a compound targeted to at least a portion of a nucleic acid molecule encoding apolipoprotein (a) to an animal or subject for the purposes of treating a disease or for the purpose of reducing plasma levels.

Claims 18, 26, 27, 31, 34, 35, 37, and 47-49 lack novelty under PCT Article 33(2) as being anticipated by Morishita et al. (Circulation, 1998 Vol. 98:1898-1904). Morishita et al. disclose three phosphorothioate backbone ribozyme oligonucleotides, 42-base pairs in length targeted to kringle 4 of the human apolipoprotein (a) (see page 1899, Methods and Figure 1A). Morishita et al. also disclose that the expression of ribozymes targeting human apolipoprotein (a) inhibited human apolipoprotein (a) protein expression in HepG2 cells (see Figures 2A and 2B), but not plasminogen concentrations (see Figure 3A). Morishita et al. further disclose that ribozyme inhibition of human apolipoprotein abolished the mitogenic action of conditioned medium in HepG2 cells.

Claims 18, 26, 27, 31, 34, 35, 37, and 47-49 lack novelty under PCT Article 33(2) as being anticipated by McLean et al. Nature, 1987 Vol. 330:132-137.
McLean et al. disclose a synthetic 30-base oligonucleotide that spanned the breakpoint of apo(a) and plasminogen similarity in the signal peptide region (see Figure 1b at dotted underline). This synthetic oligonucleotide is reverse complementary to bases 80-109 of SEQ ID NO:3 of the instant invention. It is noted that the reverse complimentarity between the synthetic oligonucleotide disclosed by McLean et al. and nucleobases 80-109 of SEQ ID NO:3 is contiguous. Given this high degree of similarity, the synthetic

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

oligonucleotide disclosed by McLean et al. meets the structural limitations of the claimed invention and would be expected to "specifically hybridize" with a nucleic acid molecule encoding human apolipoprotein (a) as defined in the instant disclosure at page 9, lines 6-25.

Claims 18, 20-27, 31, 34-40, and 47-49 lack novelty under PCT Article 33(2) as being anticipated by Rouy et al. WO 99/35241.
Rouy et al. teach antisense nucleic acids that are capable of specifically hybridizing with a nucleic acid encoding apoliproprotein (a) and down regulating gene expression (see page 23, first full paragraph). Rouy et al. teach that preferably the antisense is at least 20 nucleobases in length (see page 23, first full paragraph). Rouy et al. also teach the antisense oligonucleotide can be modified to improve stability and specificity (see page 23, first full paragraph).

Claims 18, 20-27, 31, 34-40, and 47-49 lack novelty under PCT Article 33(2) as being anticipated by U.S. Patent No. 6,008,344.
Bennett et al. disclose a modified antisense oligonucleotide targeted to phospholipase A2 group IV with the following sequence: 5'-atagcactccttcagccc-3' (see SEQ ID NO:43). Bennett et al. further disclose that the antisense oligonucleotide targeted to phospholipase A2 group IV was effective *in vitro* (see Table 2). This antisense oligonucleotide is reverse complementary to bases 457-473 of SEQ ID NO:3 of the instant invention. It is noted that the reverse complimentarity between the antisense oligonucleotide targeted to phospholipase A2 group IV disclosed by Bennett et al. and nucleobases 457-473 of SEQ ID NO:3 is not contiguous. However, the antisense oligonucleotide targeted to phospholipase A2 group IV disclosed by Bennett et al. exhibits almost 89% local similarity to nucleobases 457-473 of SEQ ID NO:3 of the instant invention, as it contains two mismatches (see attached sequence alignment). Given this high degree of similarity, the antisense oligonucleotide targeted to phospholipase A2 group IV disclosed by Bennett et al. meets the structural limitations of the claimed invention and would be expected to "specifically hybridize" with a nucleic acid molecule encoding human apolipoprotein (a) as defined in the instant disclosure at page 9, lines 6-25. Accordingly, the antisense oligonucleotide disclosed by Bennett et al. would specifically hybridize to bases 457-473 of SEQ ID NO:4 as claimed. The burden of establishing whether the prior art oligonucleotide has the further function of inhibiting gene expression under generally any assay conditions falls to Applicant. "Where the claimed and prior art products are identical or substantially identical in structure or composition, or are produced by identical or substantially identical processes, a prima facie case of either anticipation or obviousness has been established. "When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not." Therefore, the prima facie case can be rebutted by evidence showing that the prior art products do not necessarily possess the characteristics of the claimed product. "[T]he PTO can require an Applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his [her] claimed product." Therefore, it falls to Applicant to determine and provide evidence that the antisense oligonucleotide disclosed by Bennett et al. would or would not have the additional functional limitation of "inhibiting expression" of human apolipoprotein (a) protein under generally any assay conditions.

VIII. The following observations on the clarity of the claims, description, and drawings or on the questions, are made:

Claims 26-28, 32-36, and 43-49 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. The description does not disclose the claimed invention in a manner

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY	International application No. PCT/US04/14540

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

sufficiently clear and complete for the claimed invention to be carried out by a person skilled in the art because: the disclosure, while being enabling for a method of inhibiting gene expression in cells or tissues (*in vitro*) using a composition comprising a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA, does not reasonably provide enablement for a method of inhibiting gene expression in cells or tissues (*in vivo*) using a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA. The disclosure does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims 26-28, 32-36, and 43-49 are drawn to a method of inhibiting gene expression in cells or tissues (*in vitro*) using a composition comprising a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA. Given their broadest reasonable interpretation, the claims encompass *in vivo* applicability of this method for enablement purposes.

The instant invention disclosure provides methodologies for inhibiting human apolipoprotein (a) gene expression in cells in culture (*in vitro*) using antisense nucleic acids targeted to the human apolipoprotein (a) gene (see Example I).

The unpredictability of the art of antisense therapy in general adds to the lack of enablement for the current invention. For example, Branch (TIBS, February 1998 Vol. 23, pages 45-50) addresses the unpredictability and the problems faced in the antisense art with the following statements: "A ntisense molecules and ribozymes capture the imagination with their promise or rational drug design and exquisite specificity. However, they are far more difficult to produce than was originally anticipated, and their ability to eliminate the function of a single gene has never been proven."; " To minimize unwanted non-antisense effects, investigators are searching for antisense compounds and ribozymes whose targets sites are particularly vulnerable to attack. This is a challenging quest."; "H owever, their unpredictability confounds research application of nucleic acid reagents."; "N on-antisense effects are not the only impediments to rational antisense drug design. The internal structures of target RNAs and their associations with cellular proteins create physical barriers, which render most potential binding sites inaccessible to antisense molecules."; "Y ears of investigation can be required to figure out what an 'antisense' molecule is actually doing,..."; " Because knowledge of their underlying mechanism is typically acting, non-antisense effects muddy the waters."; " Because biologically active compounds generally have a variety of effects, dose-response curves are always needed to establish a compounds primary pharmacological identity. Antisense compounds are no exception. As is true of all pharmaceuticals, the value of a potential antisense drug can only be judged after its intended clinical use is known, and quantitative information about its dose-response curve of conventional drugs, which typically span two to three orders of magnitude, those of antisense drugs, extend only across a narrow concentration range."; " Because it is very difficult to predict what portions of an RNA molecule will be accessible *in vivo*, effective antisense molecules must be determined empirically by screening large number of candidates for their ability to act inside cells."; "B inding is the rare exception rather than the rule, and antisense molecules are excluded from most complementary sites. Since accessibility cannot be predicted, rational design of antisense molecules in not possible."; and, " The relationship between accessibility to oligonucleotide (ODN) binding and vulnerability to ODN-mediated antisense inhibition *in vivo* is beginning to be explored..It is not yet clear whether *in vitro* screening techniques...will identify ODN's that are effective *in vivo.*"

Jen et al. (Stem Cells, 2000, Vol. 18:307-319) discuss antisense-based therapy and the challenges that remain before the use of antisense becomes routine in a therapeutic setting. Jen et al. discuss the advances made in the art but also indicate that more progress needs to be made in the art. In the conclusion of their review, Jen et al. assert, "G iven the state of the art, it is perhaps not surprising that effective and efficient clinical translation of the antisense strategy has remained elusive." It is also stated, " The key challenges to this field have been outlined above. It is clear that they will have to be solved if this approach to specific antitumor therapy is to become a useful treatment approach. A large number of diverse and talented groups are working on this problem, and we can all hope that their efforts will help lead to establishment of this promising form of therapy." It is clear from Jen et al. that the state of the art of antisense is unpredictable and those highly skilled in the art are working towards making the art of antisense therapy more predictable but have many obstacles to overcome.

In view of the unpredictability in the art, the disclosure as filed does not provide adequate guidance or examples that would show by correlation how one skilled in the art would practice the claimed invention over the scope claimed without having to engage in trial and error or undue experimentation. The disclosure as filed is drawn to a method of inhibiting gene expression in cells or tissues (*in vitro*) using a composition comprising a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA. Given their broadest reasonable interpretation, the claims encompass *in vivo* applicability of this method for enablement purposes. However, it is unclear how the specific cell culture (*in vitro*) data is correlated with/or representative of method of inhibiting gene expression (*in vivo*) where no specific guidance (i.e. delivery route, tissue specificity, etc.) is provided.

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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PCT/US04/14540

Supplemental Box
In case the space in any of the preceding boxes is not sufficient.

The disclosure does not provide particular guidance or particular direction for delivering a composition comprising a first oligomeric compound and a second oligomeric compound to a whole animal. The disclosure does not provide guidance for the delivery of composition comprising a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA into the target organ and target cells in an animal in quantity sufficient to inhibit gene expression. While the disclosure provides guidance to addressing antisense nucleic acid administration to cells in culture, the disclosure provides no particular nexus between the inhibition of gene expression *in vivo*, as contemplated by the disclosure. The disclosure provides no particular guidance of direction for addressing the problems of targeting, permanence and quantity of expression of the gene in question, immunogenicity, etc, for antisense targeting a gene in an animal.

Therefore, in view of the breadth of the claims and the lack of guidance provided by the disclosure as well as the unpredictability of the art, one of ordinary skill in the art at the time of the invention would have required an undue amount of experimentation to make and use the claimed invention commensurate with the full scope of the claims. Due to the lack of specific guidance in the disclosure as filed and the lack of correlation between targeting and inhibiting gene expression in cell culture and *in vivo*, one of skill in the art would require specific guidance to practice the current invention. The current disclosure does not provide such guidance to target and inhibit gene expression *in vivo* and one of skill in the art would be required to perform trial and error or undue experimentation. The quantity of experimentation required to practice the invention over the scope claimed would include the de novo determination of how to engineer and deliver composition comprising a compound targeted to apolipoprotein (a), wherein said compound inhibits the expression of apolipoprotein (a) mRNA such gene expression would be inhibited to any degree, particularly, in view of the obstacles needed to overcome to use antisense therapies as exemplified in the references discussed above.